

Nutrient availability constrains the hydraulic architecture and water relations of savannah trees

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ABSTRACT

Leaf and whole plant-level functional traits were studied in five dominant woody savannah species from Central Brazil (Cerrado) to determine whether reduction of nutrient limitations in oligotrophic Cerrado soils affects carbon allocation, water relations and hydraulic architecture. Four treatments were used: control, N additions, P additions and N plus P additions. Fertilizers were applied twice yearly, from October 1998 to March 2004. Sixty-three months after the first nutrient addition, the total leaf area increment was significantly greater across all species in the N- and the N + P-fertilized plots than in the control and in the P-fertilized plots. Nitrogen fertilization significantly altered several components of hydraulic architecture: specific conductivity of terminal stems increased with N additions, whereas leaf-specific conductivity and wood density decreased in most cases. Average daily sap flow per individual was consistently higher with N and N + P additions compared to the control, but its relative increase was not as great as that of leaf area. Long-term additions of N and N + P caused midday Ψ_L to decline significantly by a mean of 0.6 MPa across all species because N-induced relative reductions in soil-to-leaf hydraulic conductance were greater than those of stomatal conductance and transpiration on a leaf area basis. Phosphorus-fertilized trees did not exhibit significant changes in midday Ψ_L . Analysis of xylem vulnerability curves indicated that N-fertilized trees were significantly less vulnerable to embolism than trees in control and P-fertilized plots. Thus, N-induced decreases in midday Ψ_L appeared to be almost entirely compensated by increases in resistance to embolism. Leaf tissue water relations characteristics also changed as a result of N-induced declines in minimum Ψ_L : osmotic potential at full turgor decreased and symplastic solute content on a dry matter basis increased linearly with declining

midday Ψ_L across species and treatments. Despite being adapted to chronic nutrient limitations, Cerrado woody species apparently have the capacity to exploit increases in nutrient availability by allocating resources to maximize carbon gain and enhance growth. The cost of increased allocation to leaf area relative to water transport capacity involved increased total water loss per plant and a decrease in minimum leaf water potentials. However, the risk of increased embolism and turgor loss was relatively low as xylem vulnerability to embolism and leaf osmotic characteristics changed in parallel with changes in plant water status induced by N fertilization.

Key-words: Cerrado trees; foliar nutrients; hydraulic architecture; long-distance water transport; long-term fertilization effects; water relations; xylem vulnerability to embolism.

INTRODUCTION

The investment in plant parts that acquire the limiting resource is usually favoured at the expense of allocation to plant parts that have a large requirement for the limiting resource. For example, if soil nutrient availability were low, plants would allocate relatively more carbon to their roots to enhance nutrient uptake (e.g. Brouwer 1963, 1983). On the other hand, if nutrient limitations were relieved by frequent fertilizations, the pattern of biomass allocation may change, favouring the expansion of the leaf surface area to enhance CO₂ capture at the expense of underground plant parts (Gleason 1993; Giardina *et al.* 2003; Gleason & Good 2003; Tingey, Johnson & Phillips 2005).

Nitrogen or phosphorus limitations on growth are widespread in tropical regions, and are particularly severe in the Neotropical savannahs of central Brazil (Cerrado) characterized by well-drained, old oxisols. These soils are not only very nutrient deficient, and their pH and cation exchange capacities low, but their aluminum saturation levels are also high, which may affect phosphorylation of proteins and

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consequently numerous metabolic processes (Furley & Ratter 1988; Furley 1999; Haridasan 2000). Moreover, seasonal drought, high irradiances and high evaporative demand of the atmosphere are characteristic of the Cerrado region. Although Cerrado trees are able to achieve high transpiration rates and high stomatal conductance, which could potentially increase nutrient uptake, hydraulic limitations and the high evaporative demand of the atmosphere require strong diurnal and seasonal stomatal control of transpiration to maintain a favourable water balance (Meinzer *et al.* 1999; Bucci *et al.* 2005). Limitations on water uptake may thus constrain the capacity of Cerrado trees to optimize nutrient uptake, particularly during the dry season.

Because soil nutrient availability influences patterns of carbon allocation, it may also affect the hydraulic architecture, and consequently the water relations of plants. If total leaf surface area increases with increasing nutrient availability at the expenses of root extension, for example, then the leaf-specific hydraulic conductivity (k_h) may decrease. Reduced leaf-specific water transport efficiency may impose a series of constraints on the water and carbon economy of the plant, such as more negative midday leaf and stem water potential, increased risk of xylem embolism, incomplete diurnal recharge of internal water storage, partial stomatal closure and inhibition of carbon assimilation. On the other hand, Cerrado tree species may partially adjust to long-term fertilization if they exhibit phenotypic plasticity and consequently can make substantial compensatory changes.

The objectives of this study were to alleviate some of the nutrient limitations of Cerrado soils by long-term N and P fertilizations, and to explore the consequences that the resulting changes in soil nutrient availability may have on patterns of carbon allocation and functional traits related to the hydraulic architecture and water relations of five dominant Cerrado tree species. It was hypothesized that N fertilization would increase leaf surface area per plant with a concomitant decrease in leaf-specific k_h , while P fertilization would have very little effect on total leaf surface area (Cordell *et al.* 2001) and long-distance water transport capacity. Compensatory mechanisms and tradeoffs resulting from changes in soil nutrient availability and their implications for plant water balance were also assessed.

MATERIALS AND METHODS

Study site and plant material

The study was conducted in a savannah site with intermediate tree density (cerrado *sensu stricto*) at the Instituto Brasileiro de Geografia e Estatística (IBGE) Ecological Reserve, a field experimental station located 33 km south of Brasília (15°56'S, 47°53'W, altitude 1100 m). At the IBGE reserve, the average annual precipitation is about 1500 mm with a pronounced dry season from May to September (www.recor.org.br). The months of June, July and August are often devoid of precipitation. Mean monthly

temperature ranges from 19 to 23 °C with diurnal temperature ranges of 20 °C being common during the dry season. The soils are deep oxisols containing about 70% clay. The development of micro-aggregate structures (e.g. cementation by iron oxides) allows Cerrado soils to be generally porous and well drained despite their high clay content. Soil pH values were low (~4.2), and P and N levels and organic matter content were 0.4 mg kg⁻¹, and 1.9 and 4 g kg⁻¹, respectively, in the upper 50 cm of soil in the study sites before the fertilization treatments began. Soil samples were collected at 0–5, 5–10, 10–20 and 40–50 cm depth before the first fertilization. Three samples were collected per plot and mixed to form a composite sample. Samples were analysed for pH in KCl, available P using Mehlich I, total nitrogen by Kjeldahl and organic matter by Walkley-Black. Analyses were performed by the soil laboratory of the Brazilian Research Center for Agriculture in the Cerrado region (EMBRAPA). The four fertilization treatments were: control (no added fertilizer), N (100 kg ha⁻¹ year⁻¹ as ammonium sulphate), P (100 kg ha⁻¹ year⁻¹, simple superphosphate) and N plus P (100 kg N ha⁻¹ year⁻¹ + 100 kg P ha⁻¹ year⁻¹). The amounts indicated represent total amounts of N and P in the fertilizer added. The fertilizer manufacturers were Adubos Araguaia (Catalão, Go, Brazil) for ammonium sulphate, and Pirecal Filler (Catalão, Go, Brazil) for the superphosphate. Plots were located in a homogeneous cerrado *sensu stricto* stand with similar soil and vegetation characteristics. The field design consisted of 15 × 15 m plots with a buffer zone of 10 m between plots, and four plots per treatment (four treatments × four replicates per treatment = 16 plots). The treatments were randomly assigned to each plot. An additional 1-m-width border surrounding the 15 × 15 m treatment plots was also fertilized. The fertilizers were applied (sprinkled) in granular forms on the organic soil surface in October at the beginning of the wet season, and in March, before the end of the wet season, every year from October 1998 to March 2004.

Five dominant woody species with different leaf phenology were selected for the study: *Schefflera macrocarpa* (C. & S.) Seem (Araliaceae), evergreen, *Ouratea hexasperma* (St. Hil.) Baill (Ochnaceae) evergreen, *Blepharocalyx salicifolius* (H.B. & K.) Berg. (Myrtaceae) brevideciduous, *Caryocar brasiliense* Camb., (Caryocaraceae) brevideciduous and *Qualea parviflora* Mart., (Vochyseaceae) deciduous (Embrapa 1998). All species renew their leaves during the dry season with the exception of *S. macrocarpa*, which produces new leaves continuously throughout the year. The brevideciduous species are functionally evergreen because they seldom remain leafless for more than a few days. The deciduous species remains leafless for about a month depending on the severity of the dry season. *O. hexasperma* is a leaf-exchanger, in the sense that it shows progressive leaf senescence and abscission during the dry season and simultaneously produces the new leaves. Three to four individuals of similar size of each species were selected in each treatment (usually one tree per plot depending on the species distribution). The study trees were located as close to the center of each plot as possible to avoid edge effects. The

mean height (m \pm SE) and the stem diameter near the soil surface (cm \pm SE) of each of the study species before the first nutrient addition were: 2.89 \pm 0.21 m and 9.45 \pm 0.93 cm (*Q. parviflora*); 4.49 \pm 0.37 m and 9.67 \pm 0.52 cm (*B. salicifolius*); 1.73 \pm 0.09 m and 6.50 \pm 0.42 cm (*O. hexasperma*); 3.42 \pm 1.45 m and 8.76 \pm 0.87 cm (*C. brasiliense*); and 5.23 \pm 0.50 m and 7.14 \pm 0.58 cm (*S. macrocarpa*).

Total leaf area and basal area were measured during January and February (wet season) of 1999, 2000 and 2004, and during July and August (dry season) of 2003. Physiological information was obtained during the dry season of 2003, and again in the dry season of 2004 after 5 years of continuous fertilization. Leaf samples for nutrient analysis were obtained during the wet season of 2004 (January).

Sap flow and water potentials

The heat dissipation method (Granier 1985, 1987) was used to measure whole-plant sap flow during two to three consecutive days in each of three to four individuals per species per treatment during the dry season of 2003 (July and August). Briefly, a pair of 20-mm-long, 2-mm-diameter hypodermic needles (Hoppner Ltda, São Paulo, SP, Brazil), containing a copper-constantan thermocouple inside a glass capillary tube and a heating element of constantan coiled around the glass tube, were inserted into the sapwood near the base of the main stem (between 15 and 30 cm above the soil surface) in each plant. The upper (downstream) probe was continuously heated at a constant power by the Joule effect, while the unheated upstream probe served as a temperature reference. Temperature differences between the upstream and downstream probes were recorded every 10 s and 10 min averages, and were stored in solid-state storage modules (SM192; Campbell Scientific, Logan, UT, USA) connected to dataloggers (CR 10X; Campbell Scientific). The average external sapwood diameter in the stems of the individuals studied was 6.79 \pm 0.28 cm, and consequently the 20 mm probes used spanned most of the hydro-active portion of the xylem. The average cross-sectional area of active xylem in all trees studied was 30.35 cm², which implies that the average thickness of the sapwood was 31 mm.

Sap flux density was calculated from the temperature difference between the two probes using an empirical calibration (Granier 1985, 1987) re-validated for tropical trees (Clearwater *et al.* 1999). The temperature differences were corrected for natural temperature gradients between the probes (Do & Rocheateu 2002). Mass flow of sap per individual was obtained by multiplying flux density by sapwood cross-sectional area. The relationship between sapwood cross-sectional area and stem diameter was obtained by injecting dye near the base of the main stem for several individuals of each species representing a range of diameters. After 2 h, the plants were decapitated a few centimetres above the point of dye injection and the area of conducting tissue was determined from the pattern of staining by the dye as it moved in the transpiration stream. Transpiration per unit leaf area was

obtained by divided mass flow of sap by the total leaf area per plant.

Leaf water potential (Ψ_L) was measured with a pressure chamber (PMS; Corvallis, OR, USA) on three different leaves from each of the same three to four individuals per species and treatment during days on which sap flow was monitored. Leaf samples collected prior to dawn (before 0600 h), at mid-morning (0930 h), midday (1300 h) and afternoon (1700 h) were immediately sealed in plastic bags upon excision and kept in a cooler until balancing pressures were determined in the laboratory within 1 h of sample collection.

Stomatal and hydraulic conductance

A steady-state porometer (Model LI-1600; Li-Cor Inc., Lincoln, NE, USA) was used to measure stomatal conductance (g_s) on three leaves of the same plants used for sap flow measurements and leaf water potential. Typically, five to six complete sets of measurements were obtained during the course of a day when sap flow was being monitored. New, fully expanded leaves from sun-exposed areas of the trees were used for measurements. Cerrado trees tend to have crowns with very low leaf area index (LAI) (0.4 to 1.5 depending on tree density and time of season) and therefore, there is little self-shading among leaves within the crown.

The apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (G_t) was determined as

$$G_t = E / \Delta \Psi,$$

where $\Delta \Psi$ is the difference between the current Ψ_L and the Ψ of the soil, and E is the average transpiration rate per unit leaf area determined from sap flow measurements at the time of Ψ_L measurements. Soil Ψ in the rooting zone of each tree was estimated by extrapolating to $E = \text{zero}$, the $\Psi_L - E$ relationships obtained by simultaneous measurements of Ψ_L and E , from pre-dawn through mid-afternoon, for each individual (Sperry *et al.* 2002; Bucci *et al.* 2005). Each value of Ψ_L was the average of three leaves per tree and three to four different trees per species. The linear regressions fitted to the $\Psi_L - E$ relationships were all significant at $P < 0.1$. Additional information on the technique for estimating soil (in the rooting zone) can be found in Bucci *et al.* (2005). A morphological index of potential transpirational demand relative to water transport capacity was obtained for each individual fitted with sap flow sensors, by dividing the total leaf area by the sapwood area ($A_L : A_s$) at the point of the sap flow sensor insertion during the dry season of 2003.

k_h and xylem vulnerability curves

k_h was measured on three large terminal branches excised before dawn from three to four individuals of each species within the treatments in May during the wet-to-dry season transition of 2004 before a substantial amount of leaf loss had occurred. A small portion of the branch cut end was then immediately removed by re-cutting under water. The

branches were then tightly covered with black plastic bags and transported back to the laboratory with the cut ends of the branches under water. Immediately after arriving at the laboratory, stem segments of about 30 cm long were rapidly cut under water and attached to a hydraulic conductivity apparatus filled with distilled, degassed water (Tyree & Sperry 1989). Water exuding from the open end of the stem drained into graduated micro-pipettes. Following a short equilibration period, water flow, generated by a constant hydraulic head of 40 cm, was measured volumetrically. Hydraulic conductivity ($\text{kg m s}^{-1} \text{MPa}^{-1}$) was calculated as $k_h = J_v / (\Delta P / \Delta X)$, where J_v is the flow rate through the branch segment (kg s^{-1}) and $\Delta P / \Delta X$ is the pressure gradient across the segment (MPa m^{-1}). Specific hydraulic conductivity (k_s ; $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) was obtained as the ratio of k_h and the cross-sectional area of the active xylem. Leaf-specific conductivity (k_L ; $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) was obtained as the ratio of k_h and the leaf area distal to the branch segment. Previous work has shown that in many Cerrado woody species features such as k_L and minimum leaf water potential, remain relatively constant throughout the year owing in part to seasonal adjustments in total leaf area per plant (Bucci *et al.* 2005).

Hydraulic vulnerability curves were determined for terminal branches of the five species by plotting the percent loss of hydraulic conductivity (PLC) against stem Ψ . Different PLC values were obtained by allowing large branches excised in the morning to dehydrate slowly in air for different time periods (Tyree & Sperry 1989). The branch length used was longer than the longest xylem vessel measured. Measurements of vessel length were done according to Zimmerman & Jeje (1981). The longest vessel length varied from 55 cm in *S. macrocarpa* to 25 cm in *O. hexasperma*. After allowing time for partial dehydration, measurements of Ψ_L and the corresponding k_h were obtained on three to five branches per species within each treatment. Stem and leaf Ψ were assumed to be in equilibrium because the branches were slowly dehydrated. Leaf water potentials were measured on the same branch on leaves adjacent to the stem segments used for k_h determinations. Maximum hydraulic conductivity ($k_{h \text{ max}}$) was obtained by flushing the samples with filtered water at a pressure of 0.2 MPa for 15 min to remove air bubbles from embolized vessels. PLC was calculated as

$$\text{PLC} = 100(1 - k_h / k_{h \text{ max}}).$$

The relationships between PLC and Ψ_L were described by a Weibull function, which yielded R^2 values > 0.9 ($P < 0.001$).

Pressure–volume relations

Pressure–volume (P–V) curves were determined for three fully developed exposed leaves from each individual during the dry season of 2004. The leaves were cut at the base of the petiole in the field, re-cut immediately under water and the entire leaves covered with black plastic bags with the cut end in water for 2 h until measurements began. After

each determination of balancing pressure with the pressure chamber, the leaves were immediately weighed to the nearest 0.001 g, and between readings they were left to transpire freely on the laboratory bench. After all balancing pressure–weight measurements were obtained; the branches were oven-dried at 70 °C to a constant mass and weighed (Tyree & Richter 1981). Pressure readings, fresh mass at each reading, saturated mass and dry mass for each leaf were entered into a pressure–volume relationship analysis program developed by Schulte & Hinckley (1985). The symplast solute content per unit dry mass was determined as follows: tissue dry mass was subtracted from tissue fresh mass to get tissue water content which was then multiplied by symplastic water fraction to get symplastic water volume. Saturated osmotic potential was converted in osmolality by multiplying by 410 milliosmol MPa^{-1} . Osmolality was then multiplied by symplastic water volume and divided by the dry mass of the leaf sample (Tyree *et al.* 1978).

Wood density

The density and saturated water content of the terminal branch sapwood were measured using three branch cores per individual. Samples were taken with an increment borer, sealed in aluminum foil and plastic bags and taken to the laboratory. Wood density (ρ) was calculated as the sample mass (M) divided by the sample volume (V). Volume was estimated by submerging the fresh sample in a container with distilled water resting on a digital balance with a 0.001 g precision. The sample was kept submerged during measurements until saturation with the help of a very small needle. The samples did not touch the walls of the container. The sample inside the water after saturation displaced an amount of water with a similar magnitude of its volume. The volume of the sample was calculated using the weight of the displaced water and considering that the density of water is 1 g cm^{-3} .

Crown leaf area, specific leaf area (SLA) and nutrients

Ten to 50 fully expanded sun leaves, depending on the total number of leaves per plant, were collected from three to four trees per species and treatment, during the dry season of 2003 and wet seasons of 1999, 2000 and 2004. Fresh leaf area was determined with a scanner. Leaves were oven-dried (70 °C), and dry mass determined for calculation of leaf area per dry mass (SLA). Total leaf area per crown was obtained by counting the total number of leaves per plant then multiplying by the average area per leaf determined from fresh leaf samples for each tree. Most of the species studied had an average of about 1280 leaves per tree. *B. salicifolius* was the only species with a relatively large number of leaves of about 8240 leaves per tree.

Foliar N and P concentration of finely ground samples collected during the wet season of 2004 was determined, respectively, by micro Kjeldahl technique and flame

photometry at EMBRAPA. Total nutrient per plant was calculated as follows: average nutrient concentration per leaf mass was multiplied by total leaf area per plant and divided by SLA.

Statistical analysis

The SPSS 11.5 statistical package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A two-way analysis of variance (ANOVA) was applied to test the data for differences among species, for treatment differences and for interactions. All data of foliar characteristics and physiological responses to fertilization within a species were analysed for normal distribution using the Kolmogorov-Smirnov test, and one-way ANOVA was applied to test differences in means of treatments. Once it was determined that differences existed among the means, Dunnett's pairwise multiple comparison test was used to compare the significance of treatments versus control for each species.

RESULTS

Foliar nutrients, leaf and stem growth, and plant water use

After 5 years of continuous fertilization, total N and P in foliage per plant was consistently greater in N-fertilized plants across all species (Table 1). However, the differences were significant only for *B. salicifolius* and *O. hexasperma* in which total N and P in foliage of N-fertilized plants was two to three times greater than in control plants. In contrast,

P fertilizations increased total P in foliage per plant only in *B. salicifolius* and appeared to lead to slightly lower total P in the remaining four species. Furthermore, P additions appeared to inhibit N uptake relative to the control in four of the species. Foliar nutrient concentration did not significantly increase with N and P fertilizations, except in *B. salicifolius* in which N increased from 11.88 to 13.52 g kg⁻¹ with N additions, and P increased from 0.66 to 1.99 g kg⁻¹ with P additions, and in *C. brasiliense* in which P increased from 0.8 to 1.0 g kg⁻¹ with N + P additions (Table 1). Although foliar N and P concentrations were not substantially affected by the fertilization treatments, foliar N to P ratios did change with nutrient additions particularly for *B. salicifolius*, *C. brasiliense* and *S. macrocarpa* (Table 1).

Variation in leaf phenology among the five species studied had to be taken into account in order to assess the impact of fertilization on carbon and nutrient allocation to leaves. For example, new leaves are produced near the end of the dry season by deciduous and brevideciduous savannah species, and consequently maximum leaf surface area per tree is achieved during the beginning and middle of the wet season. In the case of *S. macrocarpa*, one would not expect much seasonal variation in total leaf area or a reduction in the dry season (Franco *et al.* 2005). In order to obtain the actual crown expansion in response to fertilization and to minimize the effect of seasonal variations in leaf phenology, average total leaf surface area per tree in 1999, and 1999 to 2004 leaf surface area increments observed during the middle of the wet season were measured (Table 2). In 1999, 3 months after the first fertilization

Table 1. Foliar nutrient concentrations for N and P (g kg⁻¹), total amount of nutrients in foliage per plant for N and P (g crown⁻¹) and N : P ratio in leaves for the five study species in four treatments, control (C), nitrogen (N), phosphorus (P), and nitrogen plus phosphorus (N + P), during the wet season of 2004

Species	T	N (g kg ⁻¹)	N (g crown ⁻¹)	P (g kg ⁻¹)	P (g crown ⁻¹)	N : P ratio
<i>Blepharocalyx salicifolius</i>	C	11.9 ± 0.3	4.5 ± 1.2	0.7 ± 0.01	0.24 ± 0.06	18.8 ± 0.3
	N	13.5 ± 0.4*	12.6 ± 2.8**	0.6 ± 0.02	0.59 ± 0.15	21.9 ± 0.6**
	P	11.4 ± 0.4	4.9 ± 1.0	2.0 ± 0.25*	0.81 ± 0.18**	7.1 ± 1.4***
	NP	11.2 ± 0.3	9.3 ± 2.5	0.9 ± 0.04	0.66 ± 0.15	13.4 ± 0.9***
<i>Caryocar brasiliense</i>	C	11.8 ± 0.4	9.8 ± 0.7	0.8 ± 0.02	0.63 ± 0.03	15.2 ± 0.3
	N	11.9 ± 0.4	16.7 ± 4.9	0.7 ± 0.05	1.06 ± 0.26	16.3 ± 0.7
	P	9.9 ± 0.5	5.6 ± 1.7	0.9 ± 0.05	0.51 ± 0.17	11.8 ± 0.1**
	NP	11.2 ± 0.7	8.9 ± 0.5	1.0 ± 0.04*	0.77 ± 0.05	12.3 ± 0.9*
<i>Ouratea hexasperma</i>	C	9.8 ± 0.9	4.0 ± 0.9	0.7 ± 0.02	0.26 ± 0.04	15.7 ± 1.31
	N	9.7 ± 0.5	7.1 ± 0.9	0.7 ± 0.04	0.49 ± 0.05**	14.8 ± 1.3
	P	11.5 ± 1.3	2.5 ± 0.3	0.5 ± 0.15	0.15 ± 0.02	16.2 ± 0.5
	NP	11.1 ± 1.6	4.9 ± 1.3	0.8 ± 0.01	0.35 ± 0.08	13.8 ± 1
<i>Qualea parviflora</i>	C	10.9 ± 0.4	5.3 ± 2.1	0.7 ± 0.02	0.32 ± 0.13	16.6 ± 0.3
	N	11.1 ± 0.4	8.5 ± 3.7	0.6 ± 0.03	0.48 ± 0.19	17.3 ± 0.5
	P	9.9 ± 0.6	2.9 ± 0.3	0.7 ± 0.02	0.22 ± 0.03	14.8 ± 0.8
	NP	11.2 ± 0.5	7.9 ± 2.8	0.7 ± 0.27	0.49 ± 0.15	15.8 ± 0.4
<i>Schefflera macrocarpa</i>	C	10.6 ± 0.8	16.2 ± 3.2	0.6 ± 0.15	0.94 ± 0.16	17.1 ± 0.2
	N	13.9 ± 1.2	20.8 ± 9.7	0.9 ± 0.04	1.36 ± 0.64	15.1 ± 0.2**
	P	7.9 ± 1.1	8.7 ± 3.2	0.3 ± 0.12	0.45 ± 0.18	19.3 ± 0.5**
	NP	11.2 ± 0.1	16.6 ± 4.8	0.7 ± 0.01	1.10 ± 0.33	15.3 ± 0.2**

Values are mean ± SE (*n* = 3 to 4). Significant effects of fertilization with respect to the control are indicated as **P* < 0.1 and ***P* < 0.05 (Dunnett's test).

Table 2. Average total leaf area (A_L) and basal area (BA) during January and February (wet season) of 1999 and increments of A_L and BA between the 1999 and 2004 wet seasons in the four treatments, control (C), nitrogen (N), phosphorus (P), and nitrogen plus phosphorus (N + P)

Species	Treatment	Total leaf area (m ²)		Basal area (cm ²)	
		1999	2004–1999	1999	2004–1999
<i>Blepharocalyx salicifolius</i>	C	5.0 ± 2.1	0.7 ± 0.3	11.1 ± 1.5	1.8 ± 0.2
	N	3.7 ± 1.0	12.4 ± 3.2***	9.2 ± 0.1	2.0 ± 0.0
	P	4.3 ± 0.8	0.5 ± 0.1	9.3 ± 0.7	4.6 ± 1.3**
	N + P	3.3 ± 0.6	8.7 ± 2.2**	9.1 ± 0.5	3.6 ± 0.4*
<i>Caryocar brasiliense</i>	C	4.9 ± 2.0	0.0 ± 0.1	11.2 ± 1.6	1.2 ± 0.4
	N	4.1 ± 0.1	7.3 ± 2.6*	8.5 ± 1.9	3.6 ± 0.5**
	P	3.8 ± 0.2	−0.2 ± 0.1	8.4 ± 2.5	2.9 ± 0.5*
	N + P	4.7 ± 6.9	1.6 ± 0.5	10.3 ± 1.5	2.6 ± 0.3
<i>Ouratea hexasperma</i>	C	2.8 ± 0.3	0.3 ± 0.1	6.6 ± 0.1	1.3 ± 0.1
	N	3.3 ± 1.3	2.2 ± 0.7*	8.1 ± 0.8	1.6 ± 0.4
	P	2.7 ± 0.8	−1.0 ± 0.3*	6.3 ± 0.7	2.7 ± 0.6
	N + P	3.8 ± 1.8	0.8 ± 0.4	7.2 ± 1.3	3.0 ± 0.9
<i>Qualea parviflora</i>	C	5.8 ± 2.1	−0.2 ± 0.1	10.5 ± 1.5	1.9 ± 0.6
	N	4.5 ± 1.8	7.3 ± 2.8**	9.4 ± 1.3	2.0 ± 0.9
	P	3.4 ± 1.6	−0.1 ± 0.0	11.3 ± 2.9	1.4 ± 0.8
	N + P	3.7 ± 0.4	6.4 ± 1.2**	9.6 ± 2.9	2.9 ± 0.2
<i>Schefflera macrocarpa</i>	C	1.6 ± 0.4	2.8 ± 0.6	8.8 ± 1.5	0.5 ± 0.3
	N	1.5 ± 0.2	7.4 ± 1.2*	6.9 ± 1.4	2.6 ± 0.0**
	P	1.2 ± 0.3	1.5 ± 0.3	8.3 ± 1.5	1.4 ± 0.0
	N + P	2.0 ± 0.2	4.1 ± 1.0	7.4 ± 0.3	1.3 ± 0.2

Values are means ± SE ($n = 3$ to 4). Significant effects of fertilization in relation to the control are indicated as * $P < 0.1$, ** $P < 0.05$ and *** $P < 0.01$ (Dunnett's test).

(October 1998), there were no significant fertilization-specific effects on total leaf area per tree across any of the species ($P > 0.1$). However, 63 months after the first nutrient addition, the total leaf area increment was significantly greater in nearly all the N- and the N + P-fertilized plants. For example, the total leaf area of *Q. parviflora*, increased by 7.3 and 6.4 m² in the N- and the N + P-fertilized plots, respectively, while it remained practically constant in the control and P-fertilized plots. Similar trends were observed across all species. In all cases, the increases in total leaf area between 1999 and 2004 were significant in the N treatment with respect to the control. The average basal area increment between 1999 and 2004 was greater under both N and P fertilizations, but the effects were significant in only three species (Table 2). Absolute values of basal area are presented instead of relative increments normalized with respect to initial stem size because the initial basal area within each species was similar (Table 2)

At the end of the experiment in 2004, total leaf area per tree was consistently greatest in the N and N + P treatments (Fig. 1a). However, differences with respect to the control were significant only in *B. salicifolius*, *O. hexasperma* and *C. brasiliense*. Greater total leaf area in N- and N + P-fertilized trees resulted from increases in the total number of leaves rather than increased leaf size (data not shown). On the other hand, leaf size decreased significantly in P-fertilized trees of all species (data not shown). Leaf area to sapwood area ratios were greatest in N- and

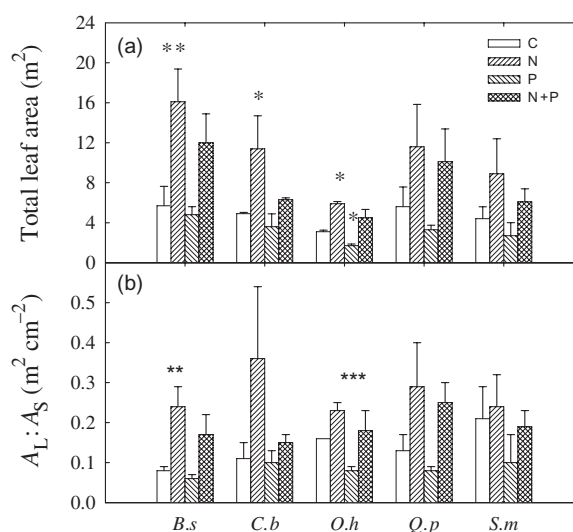


Figure 1. Average (a) total leaf area and (b) the leaf area/sapwood area ratio ($A_L : A_s$) for five Cerrado woody species in four treatments (C, control; N, nitrogen fertilization; P, phosphorous fertilization; N + P, nitrogen plus phosphorous fertilization) during the wet season of 2004. All values are mean (\pm SE) from three to four trees per species within each treatment. Significant effects of fertilization in relation to the control are indicated as * $P < 0.1$, ** $P < 0.05$ and *** $P < 0.01$ (Dunnett's test). B.s, *Blepharocalyx salicifolius*; C.b, *Caryocar brasiliense*; O.h, *Ouratea hexasperma*; Q.p, *Qualea parviflora*; S.m, *Schefflera macrocarpa*.

N + P-fertilized trees with the exception of *S. macrocarpa*, but differences with respect to the control were significant only in *B. salicifolius* and *O. hexasperma* (Fig. 1b). A two-way ANOVA was performed with nutrient treatments and species as factors, and the results suggest that all studied variables were significantly affected by fertilization ($P < 0.05$) and were different for all five species ($P < 0.005$) (results not shown). The interactions between the two factors (treatment \times species) were not significant for total canopy N and P and total leaf area per tree, but were significant at $P < 0.001$ for N and P concentrations in leaves.

Responses to nutrient additions at the whole stand level were assessed by normalizing the individual species data with respect to the control across the five dominant species. Averaging normalized data across species revealed that N additions increased leaf area (A_L) by nearly three times

relative to the control ($P < 0.001$), whereas P decreased it ($P < 0.001$) (Fig. 2a). The N-induced increase in A_L appeared to be partly inhibited when P was added to the fertilizer (Fig. 2a). Average daily sap flow per individual compared to the control (F) was consistently but not significantly higher with N and N + P additions (Fig. 2b) but its relative increase was not as great as that of leaf area. The opposite response was observed with P additions (Fig. 2b). As a consequence of larger relative increases in leaf area compared to increases in F , daily transpiration per unit leaf area (E) was lower ($P < 0.01$) in the N- and N + P-fertilized plots (Fig. 2c).

Hydraulic architecture and plant water relations

Nitrogen fertilization altered several components of the hydraulic architecture. Specific conductivity (k_s) of branches increased significantly with N additions while leaf-specific conductivity (k_L), stem xylem pressure corresponding to 50% loss of hydraulic conductivity (P^{50}) and wood density of terminal stems decreased significantly in most cases with N and N + P additions (Table 3). The leaf area to sapwood area ratio and k_s relative to the control, both increased linearly with increasing total N per crown relative to the control (Fig. 3a, b). Leaf-specific conductivity decreased by about 25% relative to the control over the range of relative variation in total crown N observed (Fig. 3c) because the approximately 2.5-fold increase in k_s was not sufficient to fully compensate for the sevenfold increase in $A_L : A_s$. Consistent with the patterns of daily transpiration derived from sap flow, stomatal conductance was typically lower in N- and N + P-fertilized plants and higher in P-fertilized plants than in control plants (Fig. 4a).

Stomatal conductance was positively correlated with total apparent soil-to-leaf hydraulic conductance (G_t) across all species and treatments (Fig. 5a). To assess whether changes in g_s , and therefore E , should have resulted in constant midday Ψ_L as G_t declined, the data were normalized with respect to their maximum values (inset in Fig. 5a). For Ψ_L to remain constant, the slope of the relationship between normalized g_s and normalized G_t should not be statistically different from one (dashed line). The value of the slope (0.33) indicated that for a 10% decline in G_t there would only be a 3% decline in g_s , and therefore a 3% or smaller decline in transpiration depending on leaf-atmosphere coupling. This suggested that minimum Ψ_L should have fallen as G_t declined. Consistent with this prediction, midday Ψ_L tended to increase sharply then asymptotically with increasing G_t (Fig. 5b). The largest change in plant-water relations associated with the long-term fertilization with N and N + P was thus a large decrease in midday Ψ_L (Fig. 4b). For example, midday Ψ_L was -1.4 MPa in *Q. parviflora* trees in the control plots, and -2.1 and -2.2 MPa in the N and N + P plots, respectively. The N-induced changes in midday Ψ_L were statistically significant in all species. P-fertilized trees did not exhibit significant changes in midday Ψ_L .

Analysis of xylem vulnerability curves indicated that 50% loss of hydraulic conductivity was attained at more

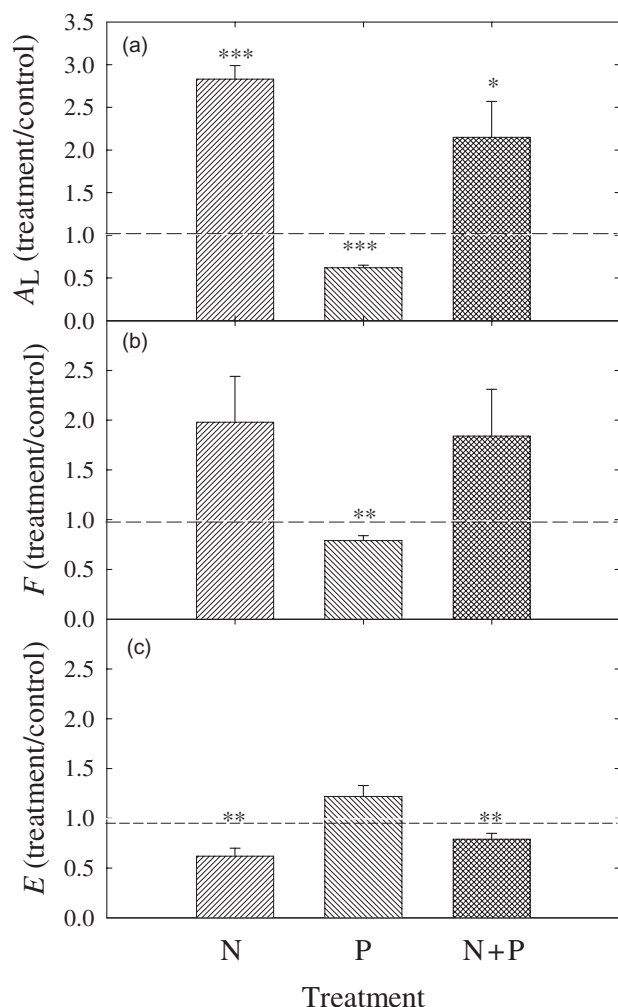


Figure 2. Pooled data for the five study species showing mean relative responses of (a) total leaf area (A_L), (b) daily water use per tree (F) and (c) daily transpiration per unit leaf surface (E) to the fertilization treatments during the dry season of 2003. Data were obtained by normalizing the individual species data with respect to the control. Values are means \pm 1SE ($n = 5$ species per treatment). Significant effects of fertilization are indicated as * $P < 0.1$, ** $P < 0.05$ and *** $P < 0.01$ (Dunnett's test).

Table 3. Average specific hydraulic conductivity (k_s), leaf-specific hydraulic conductivity (k_L), xylem pressure corresponding to 50% loss of hydraulic conductivity (P^{50}), early morning native percent loss of hydraulic conductivity (PLC) and wood density

Species	Treatment	k_s ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$)	$k_L \times 10^{-4}$ ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$)	P^{50} (MPa)	PLC (%)	Wood density (g cm^{-3})
<i>Blepharocalyx salicifolius</i>	C	2.28 \pm 0.32	7.76 \pm 0.29	-1.72 \pm 0.02	14 \pm 1	0.49 \pm 0.02
	N	5.37 \pm 0.15***	6.82 \pm 1.37	-2.30 \pm 0.06**	31 \pm 1***	0.31 \pm 0.02***
	P	2.05 \pm 0.35	7.57 \pm 1.57	-1.93 \pm 0.03*	21 \pm 1*	0.53 \pm 0.05**
	NP	4.73 \pm 0.53***	6.70 \pm 1.03	-2.17 \pm 0.03***	29 \pm 2***	0.33 \pm 0.09***
<i>Caryocar brasiliense</i>	C	2.67 \pm 0.09	10.78 \pm 0.33	-1.48 \pm 0.02	12 \pm 1	0.35 \pm 0.01
	N	3.88 \pm 0.12*	8.77 \pm 0.37***	-1.97 \pm 0.02***	24 \pm 0***	0.38 \pm 0.03**
	P	2.78 \pm 0.06	11.87 \pm 0.77	-1.62 \pm 0.06	13 \pm 1	0.34 \pm 0.08
	NP	3.50 \pm 0.22*	7.56 \pm 0.70***	-2.03 \pm 0.06***	14 \pm 1	0.35 \pm 0.02
<i>Ouratea hexasperma</i>	C	0.96 \pm 0.07	8.24 \pm 0.88	-1.48 \pm 0.02	16 \pm 6	0.46 \pm 0.02
	N	1.69 \pm 0.40*	6.29 \pm 0.59	-2.03 \pm 0.03***	33 \pm 1**	0.41 \pm 0.11***
	P	1.11 \pm 0.09	9.87 \pm 0.43	-1.48 \pm 0.04	20 \pm 1	0.42 \pm 0.03**
	NP	1.62 \pm 0.34	6.74 \pm 1.22	-1.87 \pm 0.09***	29 \pm 1**	0.42 \pm 0.05**
<i>Qualea parviflora</i>	C	0.94 \pm 0.04	5.80 \pm 0.23	-1.65 \pm 0.03	21 \pm 1	0.48 \pm 0.07
	N	1.70 \pm 0.20**	3.64 \pm 0.87*	-2.50 \pm 0.08***	30 \pm 2**	0.45 \pm 0.01*
	P	1.15 \pm 0.19	8.57 \pm 1.05**	-1.72 \pm 0.04	25 \pm 1	0.48 \pm 0.03
	NP	1.26 \pm 0.12	5.75 \pm 0.70	2.23 \pm 0.07***	30 \pm 1**	0.46 \pm 0.08
<i>Schefflera macrocarpa</i>	C	2.14 \pm 0.10	6.66 \pm 0.27	-1.72 \pm 0.02	16 \pm 2	0.56 \pm 0.09
	N	3.23 \pm 0.17*	5.50 \pm 0.32	-2.17 \pm 0.08*	16 \pm 1	0.46 \pm 0.03***
	P	0.96 \pm 0.16**	7.12 \pm 0.06	-1.77 \pm 0.02	20 \pm 1	0.59 \pm 0.06**
	NP	2.67 \pm 0.09	3.89 \pm 0.38**	-2.43 \pm 0.07**	20 \pm 1	0.56 \pm 0.01

Values are means (\pm SE) of three to four individuals per species and treatment. Significant effects of fertilization with respect to the control are indicated as * $P < 0.1$, ** $P < 0.05$ and *** $P < 0.01$ (Dunnett's test).

negative water potentials in all five species in the N- and N + P-fertilized plots compared to the control plots (Table 3, Fig. 4c), suggesting that N-fertilized trees were less vulnerable to embolism than trees in control and P-fertilized plots. There was a positive, significant correlation ($P < 0.001$) between the increments in stem xylem pressure corresponding to 50% loss of hydraulic conductivity with respect to the control (ΔP^{50}) and the increments in midday Ψ_L with respect to the control (Fig. 6). N-induced reductions in midday Ψ_L appeared to be almost entirely compensated by changes in resistance to embolism because the regression line in Fig. 6 did not differ significantly from the 1:1 relationship. However, native PLC measured prior to dawn was about 5 to 15% greater in N-fertilized plants than in the controls (Table 3). Leaf-specific hydraulic conductivity decreased linearly with decreasing vulnerability of the xylem to embolism (Fig. 7a). Similarly, G_L decreased with decreasing in xylem vulnerability to embolism for all species and treatments (Fig. 7b). The two-way ANOVA results suggested that k_s , k_L , midday Ψ_L and P^{50} were significantly affected by fertilization and species factors ($P < 0.001$) (results not shown). The interactions were not significant for k_L and midday Ψ_L .

Leaf tissue water relations characteristics were also affected by long-term nutrient additions. The bulk leaf osmotic potential at full and zero turgor generally decreased, and the symplast solute content on a dry matter basis generally increased with N fertilization (Table 4, Fig. 4d). Osmotic potential at full turgor and symplast solute content on a dry matter basis (N_s/DM) were both linearly

related to midday Ψ_L (Fig. 8). The changes in N_s/DM with Ψ_L indicated that the changes in osmotic potential were associated with true osmotic adjustment defined as a net increase in symplast solute content. The two-way ANOVA results suggested that the osmotic potential at full and zero turgor and N_s/DM were significantly affected by fertilization and species factors ($P < 0.001$) (results not shown).

DISCUSSION

Carbon allocation and growth

The results of this study show a clear effect of N fertilization on aboveground carbon allocation. Both total leaf surface area and basal area per tree increased substantially when N limitations in this savannah ecosystem were alleviated. The effects of N fertilization on aboveground carbon allocation appeared to be larger for leaves and the branches supporting them than for the main stem. Although N-induced increases in total leaf area between 1999 and 2004 were significant in all species, basal area increments in N-fertilized plants were significant only in *C. brasiliense* and *S. macrocarpa*. Allocation theory suggests that alleviating nutrient limitations for plant growth should shift the relative allocation of carbon away from roots to leaves and stems, where photosynthate is used to enhance light and CO_2 capture (Cannell & Dewar 1994; McCaughay & Coleman 1999). This change in the carbon allocation pattern should occur unless other resources are limiting. Several studies have shown that N fertilization increases aboveground primary production more than belowground

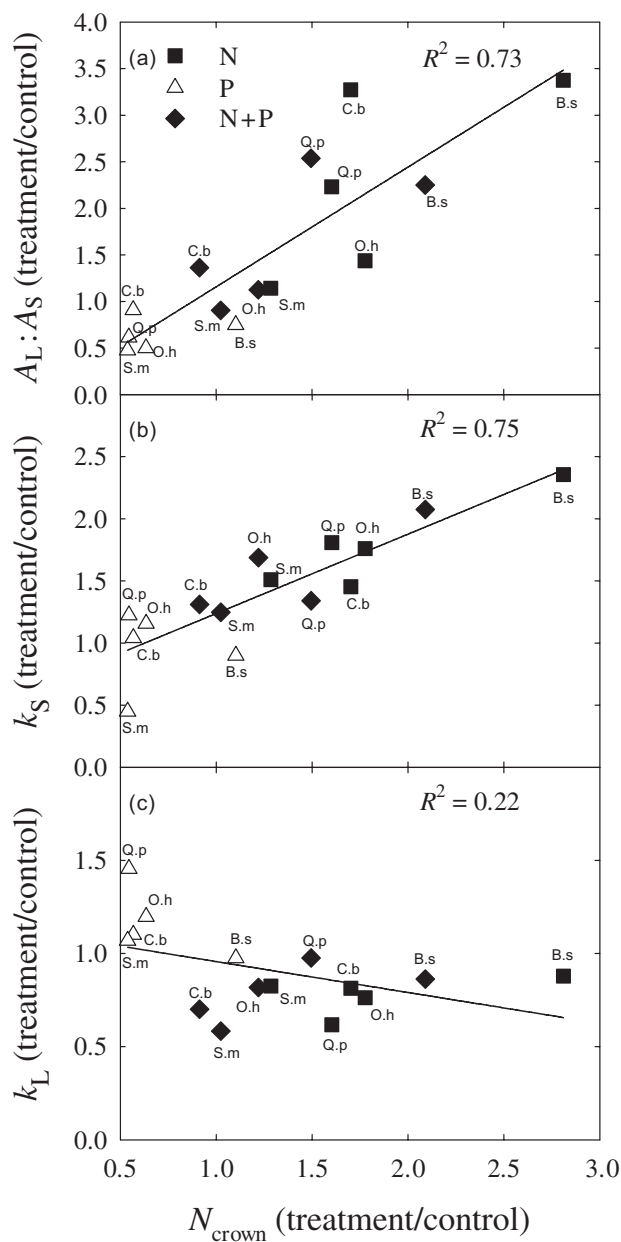


Figure 3. Relative response of (a) the leaf area/sapwood area ratio ($A_L:A_S$), (b) specific conductivity (k_S) and (c) leaf specific conductivity (k_L) to relative changes in total nitrogen per crown (N_{crown}) in the five study species. Each point represents the mean value of three to four trees per species within each treatment. The lines are linear regressions fitted to the data, (a) $y = 1.28x - 0.13$, $P < 0.0001$, (b) $y = 0.64x + 0.60$, $P < 0.0001$, (c) $y = -0.17x + 1.12$, $P < 0.1$. Symbols correspond to species responses under the nitrogen (■), phosphorus (△) and N + P (◆) treatments. B.s, *Blepharocalyx salicifolius*; C.b, *Caryocar brasiliense*; O.h, *Ouratea hexasperma*; Q.p, *Qualea parviflora*; S.m, *Schefflera macrocarpa*.

carbon allocation (Gleason 1993; Ryan *et al.* 1996; Giardina *et al.* 2003). For example, 3 years of N fertilization in *Eucalyptus saligna* stands in Hawaii resulted in a 34% increase in gross primary production, which was allocated entirely aboveground while belowground carbon allocation did not differ significantly between treatments (Giardina *et al.*

2003). Belowground growth was not measured in this study, but it is likely that root production was not enhanced in the same proportion as aboveground production as a consequence of N fertilization. This lack of compensatory adjustments in above- and belowground hydraulic architecture resulted in an imbalance between water loss by transpiration and water supplied by roots as suggested by the significant decrease in midday leaf water potentials in the N and N + P treatments.

Phosphorus fertilization, on the other hand, appeared to have no effect or a slightly inhibitory effect on leaf surface area increments per tree by the end of the 6-year fertilization experiment. Phosphorus also appeared to have an

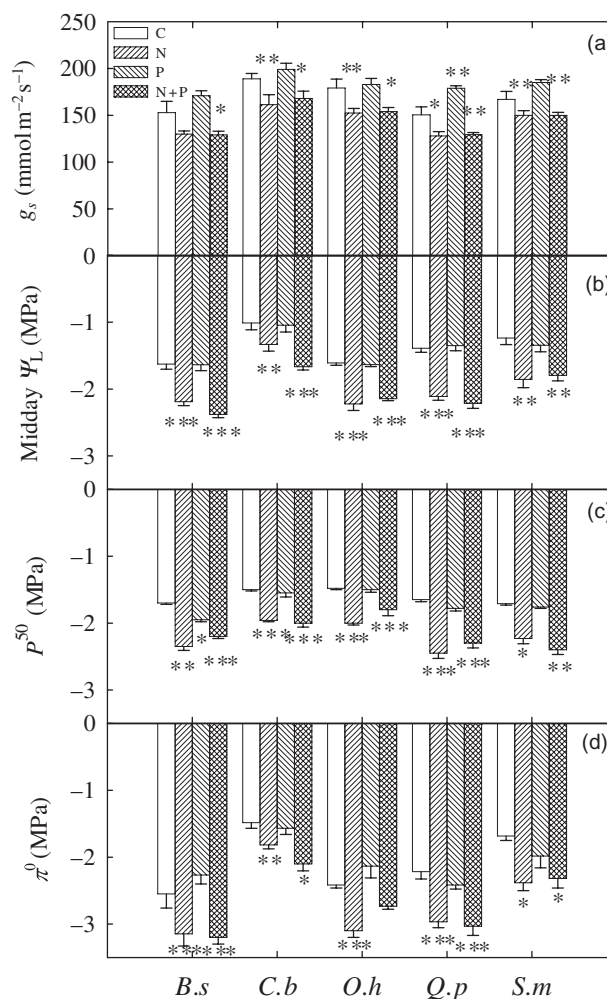


Figure 4. (a) Maximum stomatal conductance (g_s), (b) midday leaf water potentials (Midday Ψ_L), (c) stem xylem pressure corresponding to 50% loss of hydraulic conductivity (P^{50}) and (d) leaf osmotic potential at zero turgor (π^0) in the five study species under each treatment: C is control, N is nitrogen fertilization, P is phosphorous fertilization, and N + P fertilization, during the 2003 dry season. Bars are means \pm SE of three to four trees per species within each treatment. Significant effects of fertilization with respect to the control are indicated as * $P < 0.1$, ** $P < 0.05$ and *** $P < 0.01$ (Dunnett's test). B.s, *Blepharocalyx salicifolius*; C.b, *Caryocar brasiliense*; O.h, *Ouratea hexasperma*; Q.p, *Qualea parviflora*; S.m, *Schefflera macrocarpa*.

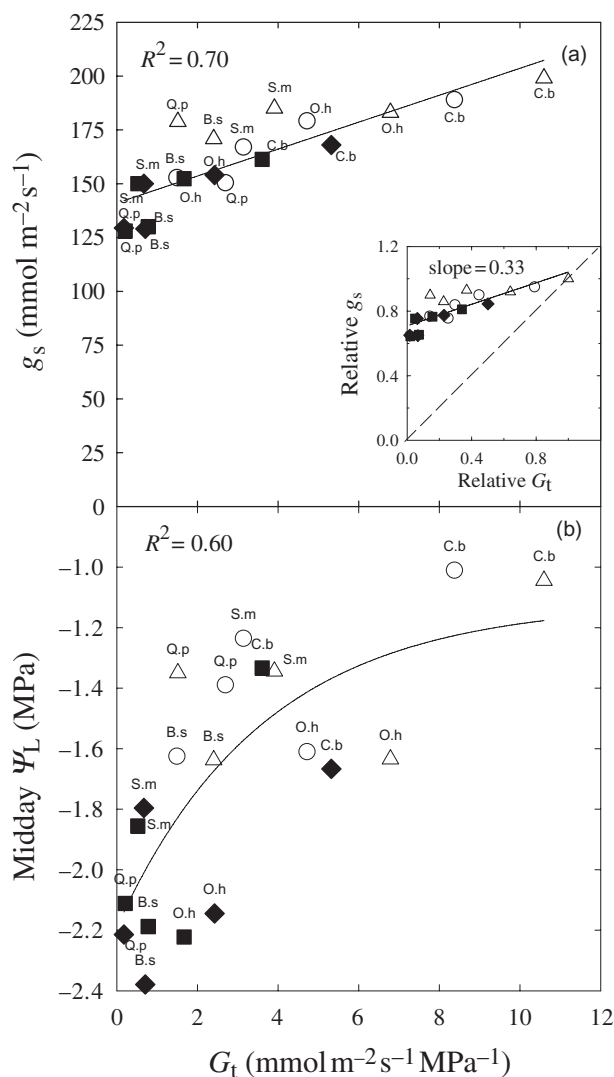


Figure 5. (a) Mean midday stomatal conductance (g_s) in relation to apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (G_t) at midday for five Cerrado tree species during the 2003 dry season. The line is a linear regression fitted to the data ($y = 141x + 6.25$, $P < 0.0001$). The inset shows the same data but normalized with respect to the maximum values. (b) Midday leaf water potential (Ψ_L) in relation to apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (G_t) at midday. The curve describes an exponential rise to a maximum fitted to the data [$y = -2 + (1 - \exp^{-0.3x})$, $P < 0.001$]. Each point represents the mean of three to four trees per treatment and species and three leaves per tree for Ψ_L . Symbols represent species responses in the control (○), nitrogen (■), phosphorus (△) and N + P (◆) treatments. B.s, *Blepharocalyx salicifolius*; C.b, *Caryocar brasiliense*; O.h, *Ouratea hexasperma*; Q.p, *Qualea parviflora*; S.m, *Schefflera macrocarpa*. The dashed line of the inset indicates a 1 to 1 relationship between the y and x variables.

inhibitory effect on total leaf area production in the N + P treatments. In all species, the increase in total leaf area per tree was lower in the N + P treatment compared to the N treatment. In previous long-term fertilization studies in native forests (e.g. Cordell *et al.* 2001), the responses of trees to additions of both N and P were not additive

compared to the responses when N and P were applied separately. The molecular and biochemical bases for this phenomenon are not known. It is possible that increasing P availability may result in an enhancement of the carboxylation capacity and consequently more foliar nitrogen has to be used in photosynthetic enzymes that could otherwise be used in leaf expansion (Cordell *et al.* 2001).

Evidence from experiments with N- and P-deficient plants suggests that contrasting morphological responses to N and P supply could be mediated by cytokinins (e.g. Forde 2002; De Groot *et al.* 2003). Increased N concentrations in the rooting medium stimulate cytokinin production and their transport from roots to shoots and stimulate biomass allocation to the shoot. Cytokinin production is less influenced by the P concentration than by the N concentration. In addition, species interactions could interfere in the responses of individual species to fertilization. In mixed vegetation, addition of the growth-limiting nutrient typically stimulates growth in only a few of the species, which perhaps sequester some of the limiting nutrient when it is added (Gussewell, Koerselman & Verhoeven 2003).

Foliar nutrients

The total amount of leaf N per crown increased two to three times in the N-fertilized trees and to a lesser extent in the

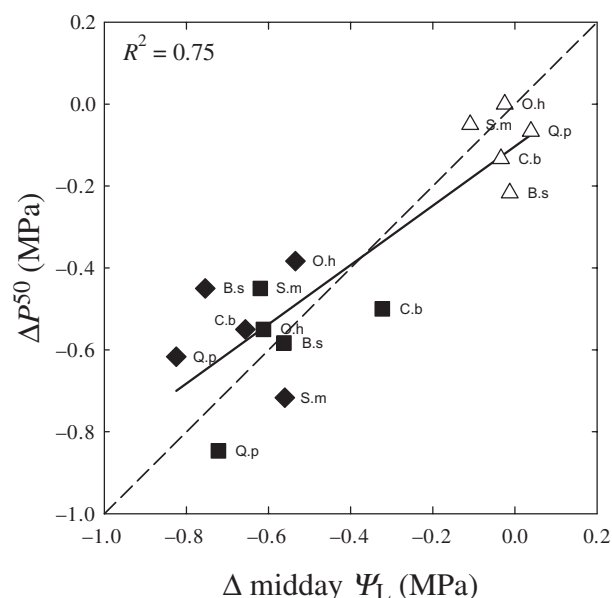


Figure 6. Increments in stem xylem pressure corresponding to 50% loss of hydraulic conductivity with respect to the control (ΔP^{50}) in relation to increments in midday leaf water potential with respect to the control ($\Delta \Psi_L$). The solid line is a linear regression fitted to the data ($y = 0.55x + 0.51$, $P < 0.0001$). The dashed line indicates the 1 to 1 relationship between the y and x variable. Values are means of three to four trees per species within each treatment (three leaves per tree for $\Delta \Psi_L$ and three to five branches for ΔP^{50}). Symbols correspond to species responses under the nitrogen (■), phosphorus (△) and N + P (◆) treatments. B.s, *Blepharocalyx salicifolius*; C.b, *Caryocar brasiliense*; O.h, *Ouratea hexasperma*; Q.p, *Qualea parviflora*; S.m, *Schefflera macrocarpa*.

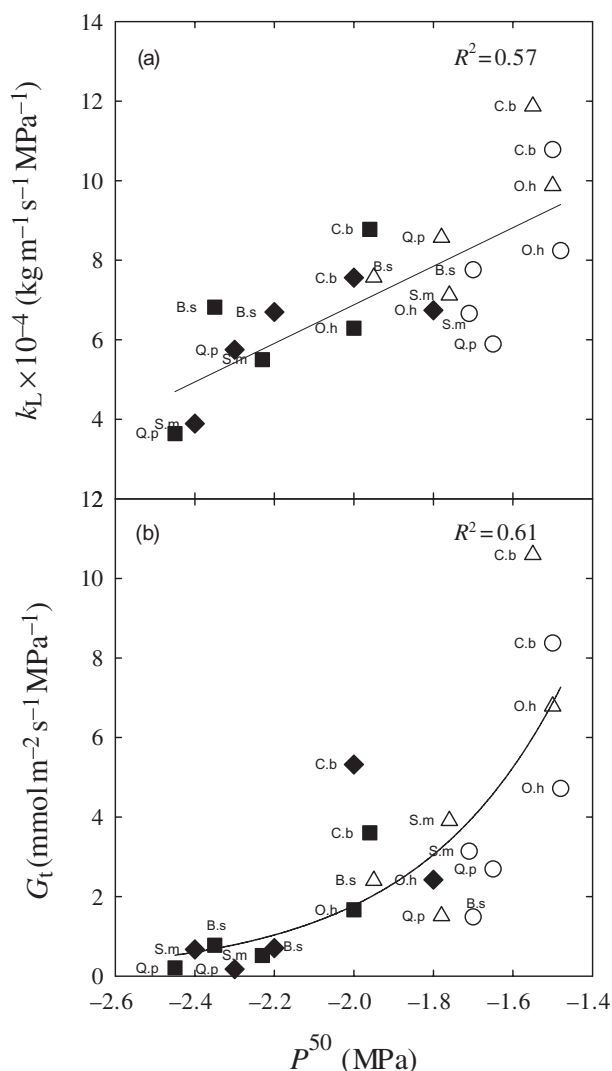


Figure 7. (a) Leaf specific conductivity (k_L) and (b) apparent leaf area-specific hydraulic conductance of the soil/root/leaf pathway (G_L) at midday in relation to xylem pressure corresponding to the 50% loss of hydraulic conductivity (P^{50}). The solid line in (a) is a linear regression fitted to the data ($y = 4.8x + 16.6$) and in (b) is an exponential growth function fitted to the data [$y = 398 \exp(2.7x)$]. Values are means of three to four trees per species within each treatment. Symbols correspond to species responses under the nitrogen (■), phosphorus (△) and N + P (◆) treatments. B.s., *Blepharocalyx salicifolius*; C.b., *Caryocar brasiliense*; O.h., *Ouratea hexasperma*; Q.p., *Qualea parviflora*; S.m., *Schefflera macrocarpa*.

N + P-fertilized trees. The increase in total N per crown resulted from a larger total leaf surface area and not from an increase in leaf N concentration, which in general was not significantly different between plants from fertilized and control plots. Therefore, we have used total N per plant rather than foliar N concentration to assess the effects of N fertilization on plant performance. On the other hand, N to P ratios in leaves differed among species after fertilization suggesting that there were species-specific differences in their capacity to acquire and utilize both nutrients (Gusewell 2004). Other studies in the Cerrado have shown

that the addition of nutrients to the soil does not necessarily result in an increase in foliar N or P concentrations (e.g. de Moraes 1994). It is known that although increased availability of nutrients to a nutrient-deficient plant initially increases its growth rate, foliar nutrient concentration may exhibit little change because of a dilution effect caused by increased carbon accumulation (Ulrich & Hill 1967; Tang *et al.* 1999; Gought, Seiler & Maier 2004). On the other hand, P-fertilized trees did not respond as much as the trees in the N-fertilized plots in terms of changes in growth or other physiological traits. It is possible that smaller responses to P additions can be partially explained by (1) the P added during fertilization was totally or partially sequestered by Al and Fe oxides and transformed into insoluble forms of P (Matson *et al.* 1999); (2) the additional P added in the fertilizer was absorbed by the individual trees and invested in other non-leafy plant parts and functions; that (3) even after fertilization, the levels of available P were not high enough to result in a substantial increase in growth rate; and/or that (4) microbial utilization of P reduced availability to the target plants. Accordingly, it is not possible with our results to rule out the possibility that P levels in the Cerrado soils are not as limiting as N levels appear to be.

Hydraulic architecture and plant water relations

The large increases in total leaf area per tree in the N-fertilized plots were expected to result in lower k_L unless k_S or sapwood area had increased proportionally to fully compensate for the larger transpirational demand. The results of this study clearly indicate that the significant increases in k_S observed in all species in the N-fertilized plots were not large enough to compensate for the increases in total leaf area per tree, and consequently k_L decreased by about 25% as a result of the N fertilization. Phosphorus fertilization, on the other hand, did not have a positive effect on total leaf surface area or an effect on k_S or k_L , with the exception of an increase in k_L in *Q. parviflora*. This is contrary to the findings of Lovelock *et al.* (2004) with dwarf mangroves in the Caribbean, who observed a substantial increase in hydraulic conductance when the inland mangrove zones were alleviated of P deficiencies by the addition of fertilizers.

The structural and anatomical basis for increased k_S in the N-fertilized trees is not known. However, it is likely that the increase in k_S was the result of an increase in the average diameter of xylem vessels. This would greatly increase the hydraulic conductivity per cross-sectional area of stem because flow through capillaries increases as a function of the radius raised to the fourth power (Tyree & Ewers 1991). This hypothesis is supported by the overall decrease in wood density in N-fertilized trees, suggesting that there were changes in sapwood anatomy that led to increased porosity with N fertilization.

Because the increase in total sap flow per individual was not proportional to the increase in total leaf area per plant in this study, transpiration rates per unit of leaf surface tended to decrease in the N-fertilized trees. Furthermore,

Table 4. Leaf tissue water relations characteristics obtained from pressure–volume relationships for the five study species in four treatments, control (C), nitrogen (n), phosphorus (p), and nitrogen plus phosphorus (N + P)

Species	Treatment	Leaf osmotic potential at full turgor, π^{100} (MPa)	Leaf osmotic potential at zero turgor, π^0 (MPa)	Symplast solute content on a dry matter basis (mOsmol g ⁻¹)
<i>Blepharocalyx salicifolius</i>	C	-2.20 ± 0.06	-2.55 ± 0.21	0.75 ± 0.06
	N	-2.63 ± 0.11***	-3.15 ± 0.18***	1.10 ± 0.14**
	P	-1.96 ± 0.04	-2.27 ± 0.13	0.72 ± 0.06
	NP	-2.60 ± 0.03***	-3.20 ± 0.10***	0.92 ± 0.02
<i>Caryocar brasiliense</i>	C	-1.35 ± 0.08	-1.48 ± 0.08	0.50 ± 0.04
	N	-1.39 ± 0.13	-1.82 ± 0.06**	0.49 ± 0.10
	P	-1.27 ± 0.13	-1.57 ± 0.09	0.31 ± 0.08
	NP	-1.85 ± 0.15**	-2.10 ± 0.10*	0.70 ± 0.10***
<i>Ouratea hexasperma</i>	C	-1.97 ± 0.15	-2.42 ± 0.04	0.87 ± 0.09
	N	-2.41 ± 0.06**	-3.10 ± 0.10***	1.40 ± 0.39***
	P	-1.62 ± 0.14	-2.13 ± 0.18	0.54 ± 0.12
	NP	-2.02 ± 0.12	-2.73 ± 0.04	0.57 ± 0.09
<i>Qualea parviflora</i>	C	-1.75 ± 0.03	-2.22 ± 0.11	0.78 ± 0.04
	N	-2.27 ± 0.08*	-2.97 ± 0.09***	1.24 ± 0.14**
	P	-1.76 ± 0.17	-2.42 ± 0.06	0.43 ± 0.05
	NP	-2.40 ± 0.13*	-3.03 ± 0.14***	1.27 ± 0.17*
<i>Schefflera macrocarpa</i>	C	-1.53 ± 0.09	-1.68 ± 0.07	0.46 ± 0.07
	N	-2.28 ± 0.15*	-2.38 ± 0.12*	1.19 ± 0.12*
	P	-1.77 ± 0.12	-1.98 ± 0.18	0.64 ± 0.09
	NP	-1.90 ± 0.08	-2.32 ± 0.15*	0.78 ± 0.06

Values are means (± SE) of three to four individuals per species and treatment. Significant effects of fertilization with respect to the control are indicated as * $P < 0.1$, ** $P < 0.05$ and *** $P < 0.01$ (Dunnett's test).

stomatal conductance tended to be lower in the N-fertilized trees, consistent with total sap flow not increasing in a 1:1 ratio with leaf area per tree. A reduction in g_s in response to fertilization was also observed by Ewers, Oren & Sperry (2000) in *Pinus taeda* and by Amponsah *et al.* (2004) in *Pinus contorta*. Stomatal conductance was positively correlated with total apparent soil-to-leaf hydraulic conductance (G_t) across all species and treatments. The slope of the relationship between normalized g_s and normalized G_t was substantially lower than one, indicative of a relatively loose coordination between gas and liquid phase conductance and of anisohydric rather than isohydric behaviour (Hubbard *et al.* 2001) as N availability varied. Indeed, the most notable impact of the long-term fertilization with N and N + P on plant–water relations in this study was a large decrease in midday Ψ_L in all species.

Substantially lower midday Ψ_L in the N-fertilized trees implies that tension in the stem xylem was greater, and that these trees may have been more susceptible to embolism formation than control trees. However, vulnerability curve data indicated that anatomical and structural changes in the xylem of N-fertilized trees caused their terminal branches to be less vulnerable to embolism than control trees. Interestingly, increased resistance to embolism in branches of N-fertilized trees was achieved despite concurrent reductions in wood density, contrary to expectations from surveys showing positive correlations between wood density and resistance to embolism across a broad range of species and wood density (Hacke *et al.* 2001).

Data obtained on many species (e.g. Sperry *et al.* 1994; Pockman & Sperry 2000; Maherali, Pockman & Jackson 2004) point to an approximate correspondence between their vulnerability to embolism and their drought resistance. In the present study, however, plant water balance was altered by N-induced changes in plant hydraulic architecture rather than changes in soil water availability. Despite lower vulnerability to embolism in the N-fertilized trees and that the fertilization-induced reductions in midday Ψ_L were almost totally compensated by increases in resistance to embolism, the N-fertilized trees exhibited a greater degree of native embolism in the early morning compared to control trees. It is possible that although P^{50} was displaced to more negative values in the N-fertilized plants, stem xylem pressures were low enough to provoke small amounts of additional embolism that was not completely reversible. We have recently studied the dynamic changes in hydraulic conductivity in leaf petioles of two savannah tree species and concluded that embolism formation and repair are two distinct phenomena with the degree of embolism being a function of tension, but the rate of refilling being a function of internal pressure imbalances (Bucci *et al.* 2003). The relationships between the vulnerability to embolism as measured by vulnerability curve methodology and the dynamics of embolism formation and refilling need to be further examined both theoretically and empirically.

The changes in leaf tissue water relations characteristics caused by long-term N additions apparently were not a direct response to higher N availability, but a water stress

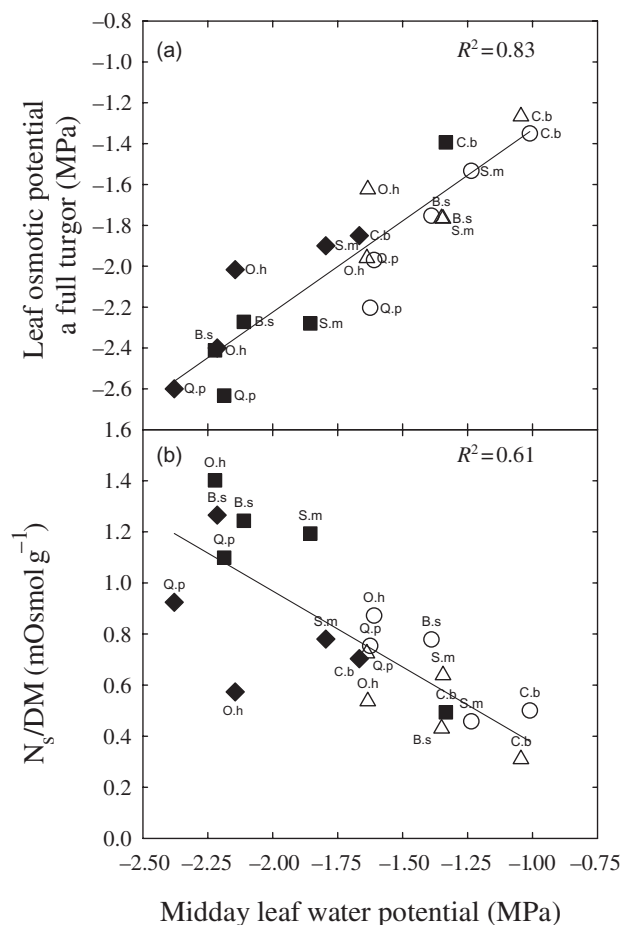


Figure 8. (a) Leaf osmotic potential a full turgor and (b) symplast solute content on a dry matter basis (N_s/DM) in relation to midday leaf water potential. The lines are the linear regressions fitted to the data. (a) $y = 0.89x - 0.44$, $P < 0.0001$, and (b) $y = -0.59x - 0.22$, $P < 0.0001$. Values are means of three to four trees per species within each treatment (three leaves per tree). Symbols correspond to species responses under the nitrogen (■), phosphorus (△) and N + P (◆) treatments. B.s, *Blepharocalyx salicifolius*; C.b, *Caryocar brasiliense*; O.h, *Ouatea hexasperma*; Q.p, *Qualea parviflora*; S.m, *Schefflera macrocarpa*.

response to maintain leaf water balance and turgor at more negative values of Ψ_L . Thus, the bulk leaf osmotic potential at full and zero turgor declined, and N_s/DM increased under N fertilization. The changes in N_s/DM indicated that the variations in osmotic potential represented true osmotic adjustment rather than a passive concentration of solutes resulting from lower tissue water content. Furthermore, P–V analyses revealed that there were no increases in leaf tissue elasticity for N- and N + P-fertilized plants (data not shown), providing additional evidence that the lower osmotic potentials at full turgor with N additions represented true osmotic adjustment. Living cells must remain turgid to be physiologically active, so osmotic adjustment can help to avoid turgor loss in leaves subjected to more negative water potential. The osmotic adjustment observed was probably the result of net accumulation of non-structural carbohydrates, inorganic solutes and other

non-nitrogenous compounds rather than nitrogenous compounds because leaf N content in N-fertilized plots did not vary with respect to the control. Other studies have also shown that the capacity for osmotic adjustment increased in plants with increasing N supply (e.g. Bennett *et al.* 1986; Garcia, Fuentes & Gallego 1996; Harvey 1997; DaMatta *et al.* 2002).

CONCLUSIONS

The more negative values of midday Ψ_L observed in the N-fertilized plots were unexpected, given the nearly isohydric behaviour of Cerrado tree species with respect to marked seasonal changes in precipitation and evaporative demand (Meinzer *et al.* 1999; Bucci *et al.* 2005). In a previous study (Bucci *et al.* 2005), we found that the isohydric behaviour with respect to minimum Ψ_L throughout the year in most Cerrado woody species was associated with deep roots to access reliable soil water sources at depth, strong stomatal control of transpiration, a decrease in total leaf surface area per tree during the dry season leading to an increase in k_L , and a tight coordination between gas and liquid phase conductance. Apparently, alleviation of N limitations affects physiological processes and patterns of carbon allocation in Cerrado woody species in a manner that prevents homeostasis of leaf water potential. Changes in k_s and g_s were not sufficient to compensate for the relative increase in leaf surface area of the N-fertilized plants and for stabilizing xylem tension in N-fertilized plants at levels similar to those in unfertilized plants. It appears that with partial or total release from N limitations, Cerrado trees allocated proportionally more resources to enhance aboveground carbon assimilation and growth than to increasing belowground biomass. Despite being adapted to chronic nutrient limitations, Cerrado woody species apparently have the capacity to exploit increases in nutrient availability by allocating resources to maximize carbon gain and enhance growth. The cost of increased allocation to leaf area relative to water transport capacity involved increased total water loss per plant and a decrease in minimum leaf water potentials. However, the risk of increased embolism and turgor loss was relatively low as xylem vulnerability to embolism and leaf osmotic characteristics changed in parallel with changes in plant water status induced by N fertilization.

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REFERENCES

- Amponsah I.G., Lieffers V.J., Comeau P.C. & Brockley R.P. (2004) Growth response and sapwood hydraulic properties of young

- lodgepole pine following repeated fertilizations. *Tree Physiology* **24**, 1099–1110.
- Bennett J.M., Jones J.W., Zur B. & Hammond L.C. (1986) Interactive effects of nitrogen and water stresses on water relations of field-grown corn leaves. *Agronomic Journal* **78**, 273–280.
- Brouwer R. (1963) Some aspects of the equilibrium between over-ground and underground plant parts. *Mededelingen Instituut Biologisch Scheikundig Onderzoek van Landbouwgewassen* **213**, 31–39.
- Brouwer R. (1983) Functional equilibrium: sense or nonsense? *Netherlands Journal of Agricultural Science* **31**, 335–348.
- Bucci S.J., Scholz F.G., Goldstein G., Meinzer F.C. & Sternberg L. D.A. (2003) Dynamic changes in hydraulic conductivity in petioles of two savanna tree species, factors and mechanisms contributing to the refilling of embolized vessels. *Plant, Cell & Environment* **26**, 1633–1645.
- Bucci S.J., Goldstein G., Meinzer F.C., Franco A.C., Campanello P. & Scholz F.G. (2005) Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plants in Neotropical savanna trees. *Trees* **19**, 296–304.
- Cannell M.G.R. & Dewar R.C. (1994) Carbon allocation in trees, a review of concepts for modeling. *Advances in Ecological Research* **25**, 59–104.
- Clearwater M.J., Meinzer F.C., Andrade J.L., Goldstein G. & Holbrook N.M. (1999) Potential errors in measurement of no uniform sap flow using heat dissipation probes. *Tree Physiology* **19**, 681–687.
- Cordell S., Goldstein G., Meinzer F.C. & Vitousek P.M. (2001) Regulation of leaf life-span and nutrient use efficiency of *Metrosideros polymorpha* trees at two extremes of a long chronosequence in Hawaii. *Oecologia* **127**, 198–206.
- DaMatta F.M., Loos R.A., Silva E.A., Loureiro M.E. & Ducatti C. (2002) Effects of soil water deficit and nitrogen nutrition on water relations and photosynthesis of pot-grown *Coffea canephora* Pierre. *Trees* **16**, 555–558.
- De Groot C., Marcelis F.L.M., van der Boogaard R., Kaiser W.M. & Lambers H. (2003) Interaction of nitrogen and phosphorus in determining of growth. *Plant and Soil* **248**, 257–268.
- Do F. & Rocheateu A. (2002) Influence of natural temperature gradients on measurements of xylem sap flow with thermal dissipation probes. 1. Field observations and possible remedies. *Tree Physiology* **22**, 641–648.
- Embrapa. (1998) *Cerrado, Ambiente e Flora*. Embrapa-CPAC, Planaltina, DF, Brazil.
- Ewers B.E., Oren R. & Sperry J.S. (2000) Influence of nutrient versus water supply on hydraulic architecture and water balance in *Pinus taeda*. *Plant, Cell & Environment* **23**, 1055–1066.
- Forde B.G. (2002) Local and long-range signaling pathways regulating plant responses to nitrate. *Annual Review of Plant Biology* **53**, 203–224.
- Franco A.C., Bustamante M., Caldas L.S., Goldstein G., Meinzer F.C., Kozovitz A.R., Rundel P. & Coradin V. (2005) Leaf functional traits of Neotropical savanna trees in relation to seasonal water deficit. *Trees* **19**, 326–335.
- Furley P.A. (1999) The nature and diversity of neotropical savanna vegetation with particular reference to the Brazilian cerrados. *Global Ecology and Biogeography* **8**, 223–241.
- Furley P.A. & Ratter J.A. (1988) Soil resources and plant communities of the central Brazilian Cerrado and their development. *Journal of Biogeography* **15**, 97–108.
- Garcia A.L., Fuentes V. & Gallego J. (1996) Influence of nitrogen supply on osmoregulation in tomato (*Lycopersicon esculentum* Mill.) plants under moderate water stress. *Plant Science* **115**, 33–38.
- Giardina C.P., Ryan M.G., Binkley D. & Fownes J.H. (2003) Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. *Global Change Biology* **9**, 1438–1450.
- Gleason S.K. (1993) Optimization of tissue nitrogen and root shoot allocation. *Annals of Botany* **71**, 23–31.
- Gleason S.K. & Good R.E. (2003) Root allocation and multiple nutrient limitations in the New Jersey Pinelands. *Ecology Letters* **6**, 220–227.
- Gough C.M., Seiler J.R. & Maier C.A. (2004) Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology. *Plant, Cell & Environment* **27**, 876–886.
- Granier A. (1985) Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. *Annales des Sciences Forestières* **42**, 193–200.
- Granier A. (1987) Evaluation of transpiration in a Douglas fir stand by mean of sap flow measurement. *Tree Physiology* **3**, 309–320.
- Gusewell S. (2004) N:P ratio in terrestrial plants: variation and functional significance. *New Phytologist* **164**, 243–266.
- Gusewell S., Koerselman W. & Verhoeven J.T.A. (2003) N:P ratio as indicators of nutrition limitation for plant population in wetlands. *Ecological Applications* **13**, 372–384.
- Hacke U.G., Sperry J.S., Pockman W.T., Davis S.D. & McCulloh K.A. (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**, 457–461.
- Haridasan M. (2000) Nutrição mineral das plantas nativas do Cerrado. *Revista Brasileira de Fisiologia Vegetal* **12**, 54–64.
- Harvey H.P. (1997) *Relationships between mineral nutrition, drought resistance and clone in Populus*. PhD dissertation, University of Victoria, Canada.
- Hubbard R.M., Ryan M.G., Stiller V. & Sperry J.S. (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell & Environment* **24**, 113–121.
- Lovelock C.E., Feller L.C., McKee K.L., Engelbrecht B.M.J. & Ball M.C. (2004) The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panama. *Functional Ecology* **18**, 25–33.
- Maherali H., Pockman W.T. & Jackson R.B. (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* **85**, 2184–2199.
- Matson P.A., McDowell W.H., Townsend A.R. & Vitousek P.M. (1999) Globalization of N deposition ecosystem consequences in tropical environments. *Biogeochemistry* **46**, 67–83.
- McConnaughay K.D.M. & Coleman J.S. (1999) Biomass allocation in plants, ontogeny or optimality? A test along three resource gradients. *Ecology* **80**, 2581–2593.
- Meinzer F.C., Goldstein G., Franco A.C., Bustamante M., Igle E., Jackson P., Caldas L. & Rundel P.W. (1999) Atmospheric and hydraulic limitations on transpiration in Brazilian cerrado woody species. *Functional Ecology* **13**, 273–282.
- de Moraes C.D.A. (1994) *Resposta de algumas espécies arbóreas nativas do Cerrado à adubação e calagem*. Master thesis, Universidade de Brasília, Brasília, Brazil.
- Pockman W.T. & Sperry J.S. (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Ecology* **14**, 1287–1299.
- Ryan M.G., Hubbard R.M., Pongracic S., Raison R.J. & McMurtrie R.E. (1996) Autotrophic respiration in *Pinus radiata* in relation to nutrient status. *Tree Physiology* **16**, 333–343.
- Schulte P.J. & Hinckley T.M. (1985) A comparison of pressure-volume curve data analysis techniques. *Journal of Experimental Botany* **36**, 590–602.
- Sperry J.S., Nichols K.L., Sullivan J.E.M. & Eastlack S.E. (1994)

- Xylem embolism in ring porous, diffuse-porous and coniferous trees of northern Utah and interior Alaska. *Ecology* **75**, 1736–1752.
- Sperry J.S., Hacke U.G., Oren R. & Comstock J.P. (2002) Water deficit and hydraulic limits to leaf water supply. *Plant, Cell & Environment* **25**, 251–263.
- Tang Z., Chambers J.L., Gudantti S. & Barnett J.P. (1999) Thinning, fertilization and crown position interact to control physiological responses of loblolly pine. *Tree Physiology* **19**, 87–94.
- Tingey D.T., Johnson M.G. & Phillips D.L. (2005) Independent and contrasting effects of elevated CO₂ and N-fertilization on root architecture in *Pinus ponderosa*. *Trees* **19**, 43–50.
- Tyree M.T. & Ewers F.W. (1991) The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**, 345–360.
- Tyree M.T. & Richter H. (1981) Alternative methods of analyzing water potential isotherms, some cautions and clarifications. *Journal of Experimental Botany* **32**, 643–653.
- Tyree M.T. & Sperry J.S. (1989) Vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Molecular Biology* **40**, 19–48.
- Tyree M.T., Cheung Y.N.S., McGregor M.E. & Talbot A.J.B. (1978) The characteristic of seasonal and ontogenic changes in the tissue-water relations of *Acer*, *Populus*, *Tsuga* and *Picea*. *Canadian Journal of Botany* **56**, 635–647.
- Ulrich A. & Hill F. (1967) Principles and practices of plant analysis. In *Soil Testing and Plant Analysis, Part II* (ed. G.W. Hardy), pp. 11–24. Science Society of America, Madison, WI, USA.
- Zimmerman U. & Jeje A.A. (1981) Vessel-length distribution of some American woody species. *Canadian Journal of Botany* **59**, 1882–1892.

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